



National Effluent EDC Screening Study

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October 2002; NERL and NRMRL requested assistance from Regional Laboratories to conduct a screening survey of 50 Municipal Sewage Treatment Plants for Endocrine Disrupting Chemicals (EDCs).

Request was for a Regional EPA inspector or State Inspector, during one of their scheduled Compliance Sampling Inspections, to collect an additional 4 gallons of water from the effluent and FEDX to NERL in Cincinnati.

Samples could be grab or composite. Some samples were collected by the WWTP personnel (ORSANCO also) after Regional Staff made contact and agreed.

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Our goal was to get a sample from a broad array of plants ranging in size 0.1 or greater *mgd* that are the following types:

- 1) Activated Sludge Plants that could be: conventional plug flow, extended aeration, oxidation ditches, contact stabilization, complete mix step aeration
- 2) Trickling Filters: Rock Media or Plastic Media
- 3) Rotating Biological Contactors
- 4) Lagoons: Aerobic or Facultative

Plant unit Types could be: with and without primary clarification, with and without effluent disinfection, aerobic or anaerobic sludge digestion, with various forms of sludge dewatering.

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Criteria for Evaluation...

If *vitellogenin* gene expression detected in male fathead minnows (*Pimephales promelas*) it would **suggest** that estrogenic compounds like the estradiol or ethynylestradiol (the active ingredient in birth control pills *and* hormone therapy) have passed through the plant.

Females normally express the *Vg* gene at sexual maturity, however, it is possible that it will be modulated in the presence of anti-estrogens or androgens in the waste water.

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Methods:

5 replicates of 2.5 liters each

1 male and 1 female per replicate

2 lab water controls: DMSO + MHRW and MHRW

1 Positive 5 ng/L EE2 Control

24 hrs at 25°C

16 hrs light – 8 hrs dark

Measured - pH, conductivity, ammonia, DO, and chlorine

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Methods Continued...

Necropsy Livers from Males and Females after 24 hrs

Place tissue in RNALater™

RNA Isolation

RT-PCR analysis

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47 Effluents tested as of March 2003

13 (27.7%) effluents up-regulated *Vg* Expression in Male Fathead Minnows

2 effluents down-regulated *Vg* Expression in Female Fathead Minnows

2 effluents were resampled and tested, demonstrated up regulation of *Vg* in Male Fathead Minnows

3 more effluents from the Chicago Metro Sewage District were tested in April 2003

2 more effluents from the Hawaiian Islands will be done in May 2003.

Samples collected from 9 out of 10 regions and in 25 States.

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FY03

- **Prepare Report of results for FY04 GPRA APM**
- **Resample all positive effluents and retest**
- **Collect 2 liters from each original site and perform chemical analyses for estrogens and androgens**

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